ANTIHYPERLIPIDEMIC ACTIVITY IN AQUEOUS LEAF EXTRACTOF *CICERACIDA* IN WISTAR ALBINO RATS

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ABSTRACT

Hyperlipidemia is a major cause of Atherosclerosis and the atherosclerosis-associated condition such as coronary heart disease ischemic cerebrovascular disease and peripheral vascular disease. The present study was carried out to investigate antihyperlipidemic properties of aqueous leaf extract of *ciceracida* (ALEC) against hyperlipidemia induced by high fat diet (HFD) in wistar albino rats. Wistar albino rats were divided in 6 groups of 6 rats in each. Group I received normal diet. Group II received high fat diet. Group III received a high fat diet supplemented with simvastatin 4mg/kg. Group IV received high fat diet supplemented with aqueous leaves extract of *ciceracida* 100 mg/kg. Group V received a high fat diet supplemented with aqueous leaves extract of *ciceracida* 250 mg/kg and Group VI received a high fat diet supplemented with aqueous leaves extract of *ciceracida* 500mg/kg for 30 days. At the end of the experiments on the 30th, day blood samples was collected 4 h after the last dose of administration using light ether anesthesia. Blood samples were collected separately from retro orbital sinus puncture into sterilized dry centrifugation tubes. Blood serum was analysed for its hypolipidemic activity. Serum analysis showed decrease cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein, atherogenic index, body weight, food intake and an increase cholesterol excretion and serum high density lipoprotein content was observed in the group III, IV, V and VI rats as compared to the group II rats, and there is no histopatholological alteration in rat aorta was seen. Thus it could be concluded that ALEC is potent antihyperlipidemic activity.

Keywords:- Lipoprotein, Hyperlipidemic diet, Obesity. CAD

No: of Tables: 3 **No: of Figures: 1** **No: of References:17**

INTRODUCTION

Hyperlipidemia is one of the risk factors for CAD. Coronary artery disease (CAD) is one of the most causes of death all over world. Data showed that 25–30% risk of CAD is reduced by treating hyperlipidemia. Although the incidence of the atherosclerosis related events has declined in the united states, these condition still accounts for the majority of morbidity and mortality among middle aged and older adults, the incidence and absolute number of annual events will increase over the next decade because of epidemic of obesity and ageing of the U.S population[1,2].

Dyslipidemia, including hyperlipidemia and hypercholesterolemia and low level of high density of lipoproteins cholesterol HDL are major cause of increased atherogenic risk; both genetic disorders and lifestyle diet high in calories, saturated fat, and cholesterol contribute to dyslipidemia. Dyslipidemia seen in developed countries around the world[3].

According to National Commission on Macroeconomics and Health (NCMH), a government of India undertaking, there would be around 62 million patients with CAD by 2015 in India and of these, 23 million would be patients younger than 40 years of age. CAD is usually due to atherosclerosis of large and medium sized arteries and Dyslipidemia has been found to be one of the most important contributing factor. As it has long been known that lipid abnormalities are major risk factors for premature CAD (National cholesterol education program2001). Severe hypertriglyceridemia (i.e. Triglyceride level of >1000mg/dl) requires therapy to prevent dyslipidemia[4]. Moderately elevated triglyceride level 150 to 400mg/dl also are concern because they often occur as part of the metabolic syndrome, which includes insulin resistance, obesity, hypertension, low HDL level and substantially increased CHD risk[5].

Medicinal plant based drug has now advantageous over modern drugs. As such are long history of use and better patient tolerance as well as public acceptance, renewable source cultivation and processing environmental friendly, local availability, plant may major source of lead generation. Several recent break through are gugulipid, taxol, artimesinin[6]. *Cicericida* is an Indian medicinal plant which is extensively used in Ayurveda and other alternative system of medicine. it is widely available in the northeast region of India specially in assam[7] Keeping these facts in view, the present study is undertaken to create a scientific base for the hypolipidemic activity on Aqueous leaves extract of *ciceracida linn* in experimental rat model.

MATERIAL AND METHODS:[8,9]

Collection of Plant materials

Leaves of *ciceracida* linn were collected from Bongaigaon distict of Assam and taxonomi were authenticated by Dr. B Doley Department of botany Goalpara College. Goalpara Assam. Leaves were

washed under tap water and were efficient dried in sun for 7 days.

Preparation of Aqueous Extraction

Leaves of *ciceracida* were sun dried, coarsely powdered and extracted by soxhelet hot extraction with water for 48 hours.

Preliminary Phytochemical screening

About 50 mg of the solvent-free extract was stirred with little quantity of dilute HCl and then filtered. The filtrate was tested for presence of various phytochemical constituents such as alkaloids, Carbohydrates, Steroids, Phenols, Tannins, Flavonoids, Glycosides and Saponins[10].

Drug and Chemicals:

Cholesterol, cholic acids (sigma chemicals, USA), *ciceracida*, simvastatin (Intas health care, Ahmadabad, India), Cholesterol, triglyceride, HDL-cholesterol estimating kits (RFCL Pvt. Ltd, Gudgeon, India), Citric acid, Sodium citrate, Dextrose, Adenosine Di phosphate (ADP), Heparin (S.D. Fine chemicals, India). High fat diet (HFD) 4% cholesterol, 1% cholic acid, 10% coconut oil

Experimental Animals:

Adults Wistar albino rats of both sexes, eight weeks old, weighing 180-200 g were used in present investigation. The animals were maintained in propylene cages in the departmental Animal House Facility with 12 hrs light and dark cycle. Temperature was maintained at 25±3 ^{0}C. Feeding schedule consisted of rat pellet diet and water ad libitum. Daily intake of food was quantitated precisely. Prior to initiation of experiments, the entire experimental protocol was submitted to the Institutional Animal Ethical Committee, reviewed and the approval obtained as per CPCSEA guidelines.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the extracts (ALEC) were administered orally at the dose of 2000 mg/kg. The animals were observed for toxic symptoms and behavioral changes continuously for the first 4 hrs after dosing. Finally, the number of survivors was noted after 24 hrs. From the next day onwards, each day 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for the next 14 days. Aqueous leaves extract of *ciceracida* were shows no mortality in rats. No toxic symptoms were observed even at the dose of 2000 mg/kg during the period of 14 days. The LD 50 value of ALEC was found to be more than 2000 mg/kg. so dose are selected randomly as 100 mg 250 mg and 500 mg.

Antihyperlipidemic activity:

The rats were divided into the following 6 groups each consist of 6 animals. The hyperlipidemia was induced by feeding hyperlipidemic diet for 30 days[11].

Group I: Received 0.5% CMC with hyperlipidemic diet for 30 days

Group II: Received only 0.5% CMC for 30 days

Group III: Simvastatin 4mg/kg/day along with hyperlipidemic diet.

Group IV: Aqueous leaf extract of *ciceracida* 100 mg/kg/day along with hyperlipidemic diet.

Group V: Aqueous leaf extract of *ciceracida* 250 mg/kg/day along with hyperlipidemic diet.

Group VI: Aqueous leaf extract of *ciceracida* 500mg/kg/day along with hyperlipidemic diet for a period of 30 days.

All animals had free access to diet and water. The daily diet consumed by animals was calculated by subtracting the leftover diet the next day from the previous day's added diet. The body weight of each animal was recorded every day.

Collection of blood samples and biochemical analysis from serum

Blood sample were collected using light ether anesthesia on 30th day after 4 hours of last dose. Samples were collected separately from retro orbital sinus puncture into sterilized dry centrifugation tubes. Samples were allowed to stand for 30 min at 37˚C. The clear serum was separated at 2500 rpm for 10 min using centrifuge. The biochemical investigation was carried out to assess total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein[12].

Histopathology

After the decapitation of the animals, the aorta were removed and fixed in 10% neutral-buffered formaldehyde solution. Fixed tissues were embedded in paraffin, cut into sections and placed on microscope slides. Slides were stained with hematoxylin and eosin for the histomorphological examination which was performed under light microscopy[13].

Statistical Analysis

Results are presented as mean ±S.D. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post test to identify significant difference. All analyses were performed using Graph Pad Prism statistical software. A level of $p < 0.001$ was considered significant.

RESULT

Preliminary phytochemical analysis of aqueous extract of ciceracida shows positive qualitative test for tannins, alkaloids, phenols, glycosides, flavonoids (especially flavones) & carbohydrates. Liberman-Buchard test for steroids showed falls positive results, so this conclude that steroids are present in very low concentration & may not contributes to effects of see aqueous extract. Tests for proteins are negative.

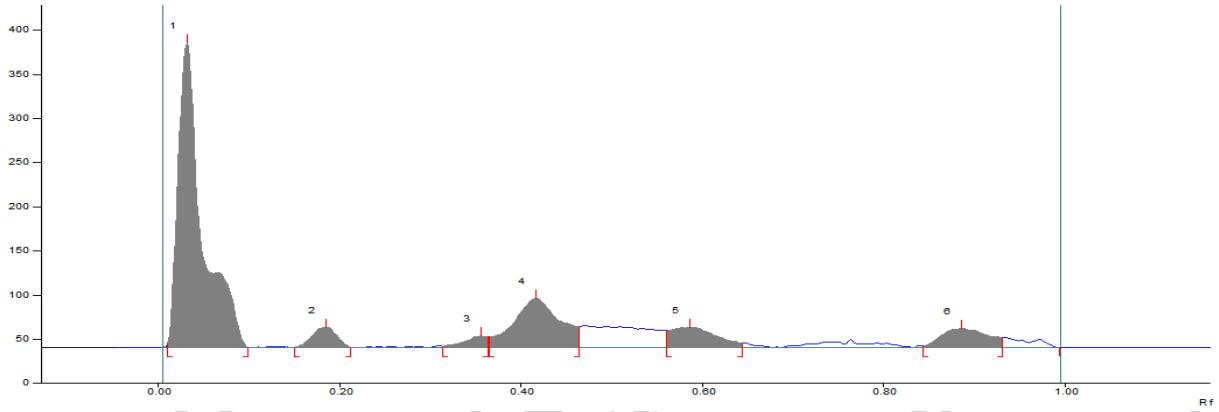
HPTLC (High performance thin layer chromatography) was performed in **S.D.M.** centre for research in ayurveda and allied sciences (ayush centre for excellence and recognized SIROS by DSIR) laxminarayana

nagar, p.o. kuthpady –udupi [karnataka]. R_f values were identified and matched with library reference values. R_f library values suggest that presence of flavonoids(querecin -3-o rutinosides, isoquerecin)

Table; A R_f values

Track 6, ID: Cicaraeida

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.2 AU	0.03 Rf	344.6 AU	71.59 %	0.10 Rf	0.2 AU	6603.1 AU	60.05 %
2	0.15 Rf	0.2 AU	0.19 Rf	23.1 AU	4.80 %	0.21 Rf	0.2 AU	452.8 AU	4.12 %
3	0.31 Rf	1.5 AU	0.36 Rf	13.0 AU	2.69 %	0.36 Rf	12.3 AU	242.7 AU	2.21 %
4	0.37 Rf	12.4 AU	0.42 Rf	56.0 AU	11.63 %	0.46 Rf	23.6 AU	2037.8 AU	18.53 %
5	0.56 Rf	19.5 AU	0.59 Rf	23.0 AU	4.78 %	0.65 Rf	4.9 AU	855.5 AU	7.78 %
6	0.85 Rf	2.3 AU	0.89 Rf	21.7 AU	4.51 %	0.93 Rf	10.9 AU	804.9 AU	7.32 %



Graph:A- R_f values

Administration of ALEC at a dose 100 mg/kg of p.o to hyperlipidemia induced animal resulted in a decreased of total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol atherogenic index and fecal cholesterol excretion and an increased in HDL cholesterol where comparing with control similarly 250 mg/kg and 500 mg/kg ALEC administered group shown more reduction in total cholesterol, triglyceride, LDL cholesterol VLDL cholesterol and atherogenic index, and fecal cholesterol excretion and an increased in HDL cholesterol was dose dependent and effective.

Table 1: Effect of aqueous leaf extract of *ciceracida* on various biochemical parameters in hyperlipidemic rats.

GROUP		PARAMETERS					
	CHOLESTEROL	TG	HDL	LDL	VLDL	A.GENIC INDEX	FECAL EXCRESTION
NORMAL	87.17±1.94	125.0±2.08	42.50±1.87	22.33±1.21	24.5±1.87	1.28±0.14	19.0±1.41
HFD	234.8±4.00	257.2±2.31	25.50±1.87	172.5±1.87	52.00±1.87	9.66±.81	22.83±1.47
SIMVASTATIN	135.7±4.63***	134.8±2.92***	56.50±1.87***	27.5±1.04***	27.83±2.31**	1.11±0.12***	25.17±1.04*
HFD+ ALEC 100 mg/kg	198.8±1.47**	252.8±1.34*	29.67±1.96**	133.7±1.63*	48.17±1.47*	7.33±0.81*	23.67±1.63
HFD+ ALEC 250 mg/kg	178.5±1.04***	165.8±1.75***	32.0±1.78***	96.5±1.63***	35.67±3.4***	5.0±0.63**	25.17±1.16*
HFD+ ALEC 500 mg/kg	144±1.47***	137.0±1.78***	50.50±1.51***	31.33±2.73***	28.67±1.36*	2.5±.54**	28.83±0.83**

Values are represent as mean± S.D (n=6) ANOVA: Dunnetts multiple comparison test as *p <0.05, **p <0.001 as compared with high fat diet group.

Table 2: Effect of aqueous leaf extract of *ciceracida* in body weight of rats animal model

Group	initial	5th day	10th day	15th day	20th day	25th day	30th day
HFD	186.3±1.5	187.3±1.0	187.8±1.4	194.5±1.64	197±1.4	197±1.6	197.5±2.3
Control	189.3±1.7	170.3±1.6	147±1.4	133.3±1.6	135.3±3.0	133.7±3.3	126.8±3.4
Simvastatin 4 mg/kg	189.7±2.6	186.5±1.5	176.2±1.1	172.3±2.94	167±1.6	161.7±2.0	155.5±1.8
ALEC 100 mg/kg	194.8±1.9	167±2.0	156.8±1.4	141.5±1.3	142.2±1.9	135.3±1.6	134.7±2.5
ALEC 250 mg/kg	194±3.1	186.7±3.3	183.5±1.8	174.5±1.7	174.5±3.6	163.2±1.9	156±1.4
ALEC 500 mg/kg	195.3±1.2	183.5±1.8	176.7±1.3	173±1.7	167.2±1.1	162.2±2.1	153.7±1.2

Values are represent as mean± S.D (n=6) ANOVA: Dunnetts multiple comparison test as *p <0.05, **p <0.001 as compared with high fat diet group.

Above data clearly indicates that significant weight increased in high diet food treated groups compared with control and significant weight reduction in ALEC treated groups compared with high food diet treated groups.

Histopathological examination

No histological alteration in rat aorta were established in any six of the animal in each groups.

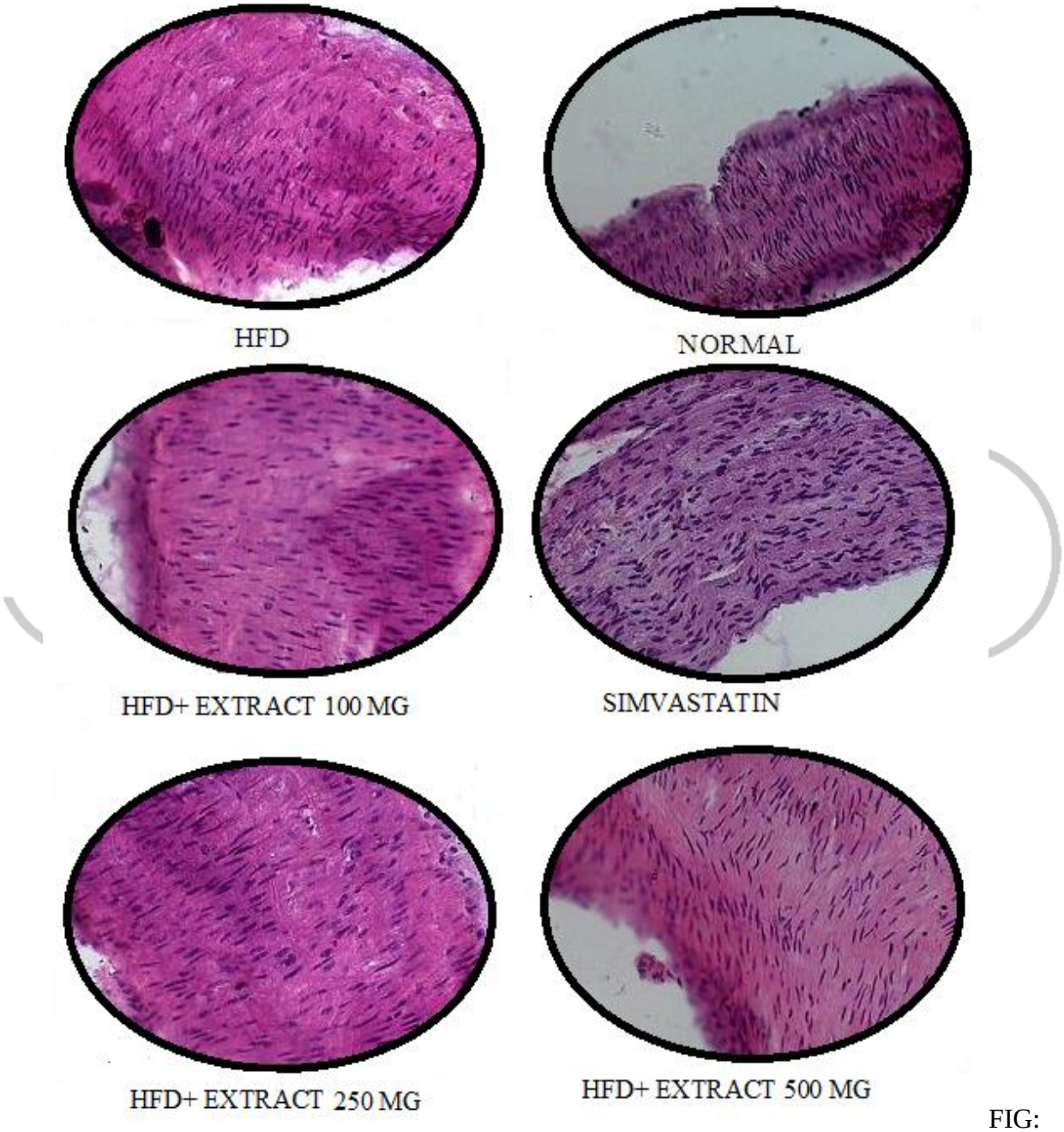


FIG:

HISTOPATHOLOGY OF AORTA OF EXPERIMENTAL RATS

DISCUSSION

The present study was carried with the aim of evaluating antihyperlipidemic activity of ALEC. Several animal and human studies have confirmed that the hyperlipidemic properties of saturated fatty acids and cholesterol which include increasing total cholesterol and altering lipoprotein pattern and whose mechanisms remain under study. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia related metabolic disturbances in different animal models. Increased cholesterol concentrations in plasma are cause of coronary atherosclerosis and increase risk of CAD[14]. High fat administration increases the biosynthesis of phospholipids possibly by a decrease in phospholipase activity or increased phospholipid turnover due to an onset of inflammatory process[15].

In this study, hyperlipidemia was induced in rats by adding high fat diet (HFD) which consist of cholesterol (4%), cholic acid (1%) and coconut oil (10%) to the diet for 30 days. Furthermore, body weights were significantly enhanced by the intake of a hyperlipidemic diet as compared to control rats, and it was accompanied by decrease body weights significantly those treated with ALEC. Determinations of the lipid profile in serum from rats fed a hyperlipidemic diet revealed higher levels of serum cholesterol and triglyceride, LDL-cholesterol, VLDL-cholesterol, Atherogenic index as compared to controls and marked decrease HDL-cholesterol in rats fed a hyperlipidemic diet as compared to control. But ALEC treated groups with hyperlipidemic diet shows significant as well as dose dependant decreased serum cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol and Atherogenic index as compared to hyperlipidemic diet and marked increased HDL-cholesterol as compared to hyperlipidemic diet[16,17].

High cholesterol diet induced hyperlipidemia is associated with alteration in the activities of enzymes responsible for cholesterol transport and metabolism. High diet couse significant increase in triglycerides level by inhibiting capillary lipoprotein lipase which is responsible for plasma triglycerides hydrolysis. Thus the rat given with high fat died significant increases triglycerides level[8].

Simvastatin is a HMG Co A reductase inhibitor which is used as standard drug. The elevation of serum cholesterol levels followed by giving high fat diet to rats was due to stimulation of 3-hydroxy-3-methylglutaryl-Co-enzyme A reductaseactivity in the liver. Aqueous leaf extract of *ciciracida* shows lowering of TG levels by increasing the lipoprotein lipase activities. The cholesterol lowering effect of ALEC was observed in rats treated with high fat diet may be due to the HMG Co A reductase inhibition.

The result of phytochemical investigation has lead to the conclusion that aqueous leaf extract of *ciceracida* contains flavonoids, glycosides, saponin. From the literature revied of antihyperlipidemic activity of herbal plants suggested that saponin and flavonoids content may be responsible for lowering of TG, TC, LDL and

VLDL levels and reduced the cardiovascular disease by increasing HDL levels. Aqueous leaf extract of *ciceracida* have ability to restore the triglycerides level by stimulating lipolytic activity of plasma lipoproteins lipase and reduction in oxidatve stress may be responsible for the antihyperlipidemic activity. However from the literature review hypothesized those medicinal plants containing flavonoids may responsible for antihyperlipidemic activity[8]. In conclusion, the aqueous leaf extract of *ciceracida* may possesses antihyperlipidemic.

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